FLUORESCENT PROBE FOR ZINC

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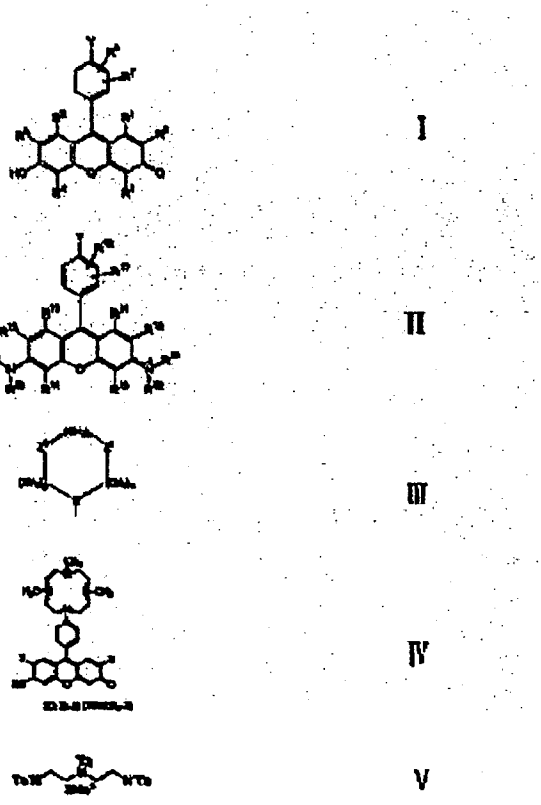
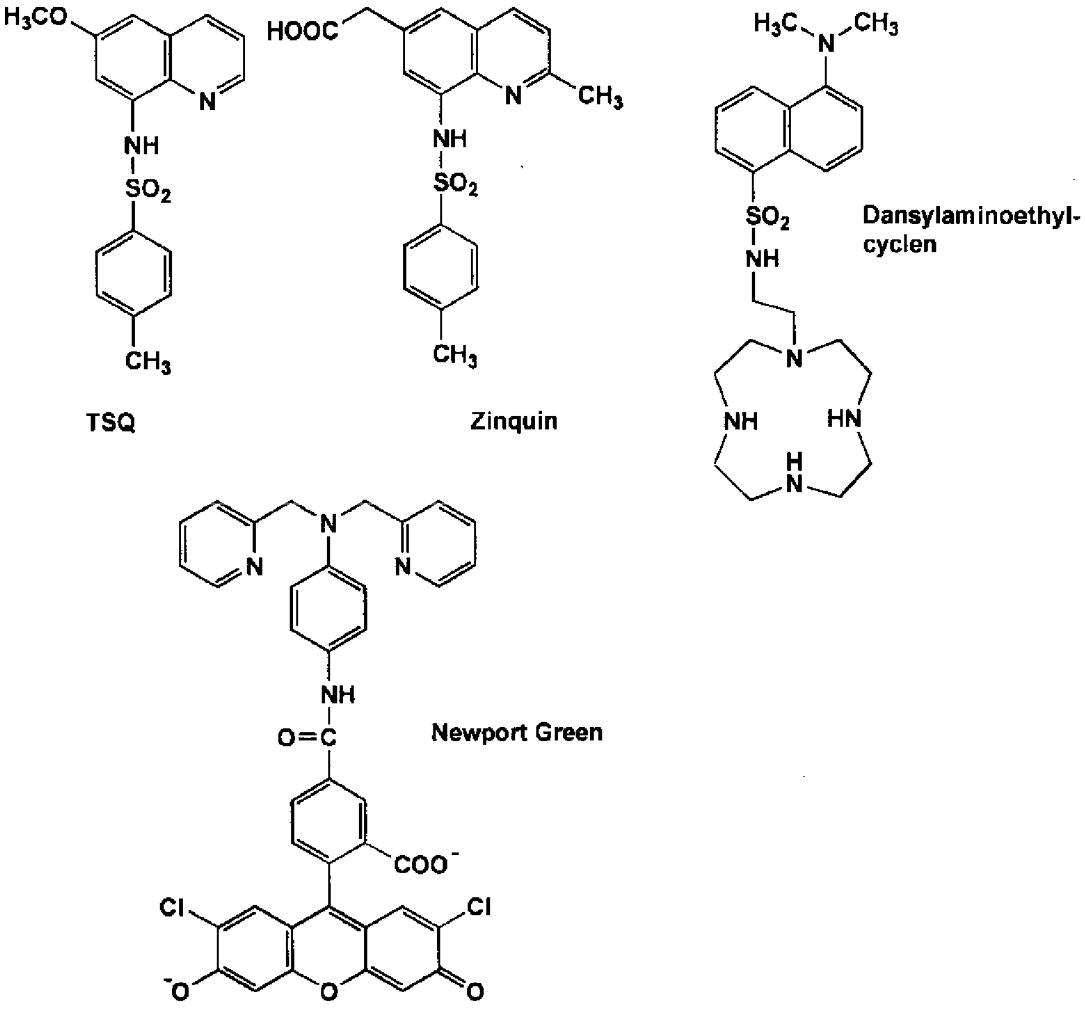
Zusammenfassung von **JP2000239272**

PROBLEM TO BE SOLVED: To obtain the subject new compound capable of specifically capturing zinc ion, forming a complex generating a strong fluorescent light by an exciting light in a long wave length range, and useful as a highly sensitive zinc probe.
SOLUTION: The compounds are expressed by formula I or II R1 to R6 are each H, a halogen, cyano or a lower alkyl; R7, R8 are each H, a halogen or the like; R11 to R16 are each H, a halogen, cyano or a lower alkyl; R17, R18 are each H a halogen or the like; R21 to R24 are each H or a lower alkyl; Y is a group of formula III [(Z1, Z2 are each O, S, N(R51) (R51 is H, lower alkyl or the like) or the like; (m), (n), (p) are each 2, 3), a group of the formula: N(R31) (R32) [R31, R32 are each the formula: (CH2) sN(R41)(R42) or the like] or the like], e.g.; a compound of formula IV. The compound of the formula I, e.g. in the case of the compound of formula IV, is obtained by performing various reactions by using N-phenyldiethanolamine and a compound of formula V as starting materials. By using the above compound, it is possible to measure zinc ion concentration in a living body in a high sensitivity without generating cytotoxicity.



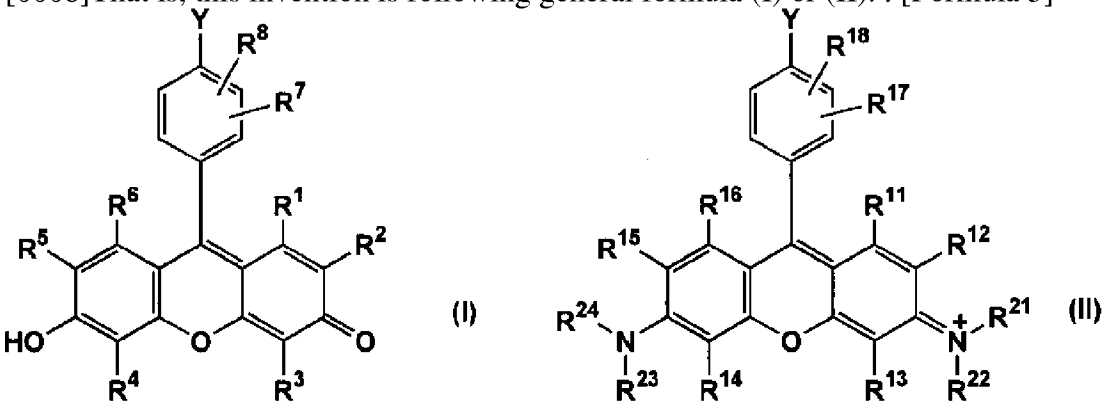
DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to the zinc fluorescent probe which catches zinc ion specifically and shows a fluorescence.
[0002]
[Description of the Prior Art]Zinc is an indispensable metallic element with many contents after iron in the human inside of the body, it combines with protein firmly and almost all intracellular zinc ion is participating in proteinic structure preserving and functional expression. There are various reports also about the physiological role of the free zinc ion (it is usually below muM level) which exists in intracellular in very small quantities very much. It is thought that zinc ion is especially concerned with the apoptosis which is one of the cell death deeply, and there is also a report of promoting formation of the senile plaque of an Alzheimer disease.
[0003]In order to measure the zinc ion of an in-house conventionally, zinc ion is caught specifically, a complex is formed and the compound (zinc fluorescent probe) which shows a fluorescence in connection with complexing is used. As a zinc fluorescent probe, for example TSQ (Reyes, J.G., et al., Biol. Res., 27, 49, 1994), Zinquin ethyl ester (Tsuda, M. et al., Neurosci., 17, 6678, 1997), Dansylaminoethylcyclen (Koike, T. et al., J. Am.Chem. Soc., 118, 12686, 1996), Newport Green (Molecular.) "Handbook of Fluorescent Probes and Research Chemicals" 6th Edition by Richard P. Haugland pp.531-540) etc. which are the catalogs of Probe. It is put in practical use.
[0004]
[Formula 4]

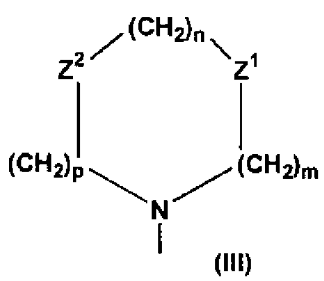


[0005]However, in the measurement using TSQ, Zinquin, or Dansylaminoethylcyclen. Since it is necessary to use the excitation light of a short wavelength region (excited wavelengths 367 nm, respectively) They are 368 nm and 323 nm. When these zinc fluorescence BUROBU is used for measurement of a living organism, There is a problem of being easy to receive the influence by the autofluorescence (fluorescence which NADH and flavins show) which excitation by short wavelength may cause a cell damage (a cell technology, 17, pp.584-595, 1998), and the cell lineage itself has in the case of measurement. The difference in the environment where, as for Dansylaminoethylcyclen, a reagent exists at the time of measurement, Namely, the water solubility in the outside of the kind of solvent, or a cell, intracellular, or a cell membrane, It has the fault that fluorescence intensity changes with the differences among environment, such as lipophilicity, a lot (protein, nucleic acid and an enzyme, a special number item, 42, pp.171-176, 1997), and since lipophilicity of TSQ is high, it also has the problem that it is difficult for the whole cell for you to make it distributed uniformly. Although Newport Green can be measured by the excitation light of long wavelength, it has the problem that an affinity with zinc ion is low and does not have practical sensitometry. Therefore, high sensitivity is asked for development of the zinc fluorescent probe which can measure zinc ion, without causing a cell damage.

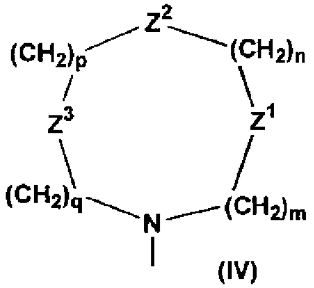
[0006]
[Problem(s) to be Solved by the Invention]There is a technical problem of this invention in providing a compound available as a high sensitivity zinc fluorescent probe, or its salt. It is a technical problem of this invention to provide a compound available as a zinc fluorescent probe which can catch zinc ion specifically, is more specifically excellent in the fluorescence intensity of the complex after prehension, and can perform fluorometry by the excitation light of long wavelength. There is another technical problem of this invention in providing the measuring method of the zinc ion using the zinc fluorescent probe containing the compound which has the above-mentioned feature, and this zinc fluorescent probe.
[0007]
[Means for Solving the Problem]As a result of inquiring wholeheartedly that this invention persons should solve the above-mentioned technical problem, it found out a compound expressed with following general formula (I) or (II) having high singularity to zinc ion, catching zinc ion, and forming a complex which shows a strong fluorescence by excitation light of a long wavelength field. Without causing a cell damage, when this compound was used as a zinc fluorescent probe, change of fluorescence intensity by difference in environment where reagents besides a kind of solvent or an in-house etc. exist was small, and found out very exact and that it could measure to high sensitivity for zinc ion in the living body. This invention is completed based on these knowledge.
[0008]That is, this invention is following general formula (I) or (II). : [Formula 5]



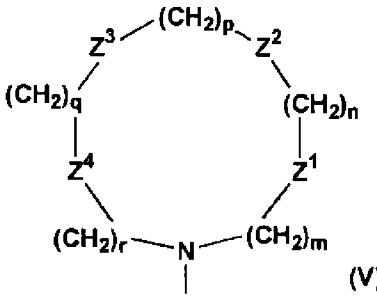
[The inside of formula (I), R^1, R^2, R^3, R^4, Independently R^5 and R^6, respectively A hydrogen atom, a halogen atom, A cyano group or a low-grade alkyl group is shown, and independently;R^7 and R^8, respectively A hydrogen atom, A halogen atom or a low-grade alkyl group is shown, and The inside of; type (II), R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, Independently R^{16}, respectively And a hydrogen atom, a halogen atom, a cyano group, A low-grade alkyl group is shown and independently;R^{17} and R^{18}, respectively Or a hydrogen atom, A halogen atom or a low-grade alkyl group is shown,;R^{21}, R^{22}, R^{23}, and R^{24} show a hydrogen atom or a low-grade alkyl group independently, respectively, and the inside of; type (I) and formula (II) and Y are following formula (III) thru/or (V). : [Formula 6]



(III)



(IV)



(V)

Although Z^1, Z^2, Z^3, and Z^4 show -N(R^{51})-, -O-, or -S- independently among [type, respectively, At least one of Z^1, Z^2, Z^3, and Z^4 shows -N(R^{51})-, a low-grade alkyl group (this amino group -- a low-grade alkyl group.) by which R^{51} was replaced by hydrogen atom, a low-grade alkyl group, 1, or two or more amino groups . It may be replaced by low-grade alkyl sulfonyl group or an aryl sulfonyl group. or -- one -- or -- two -- a piece -- more than -- a hydroxyl group -- replacing -- having had -- low-grade -- an alkyl group -- being shown --; -- m -- n -- p -- q -- and -- r -- respectively -- independent -- two -- or -- three -- an integer -- being shown --] -- expressing -- having -- a basis -- being shown -- or -- or -- a formula -- (VI) -- : - N (R^{31}) (R^{32})

The inside of [type, R^{31}, and R^{32} are formula (VII) thru/or (X) of the following [respectively] independently. : [Formula 7]

$$-(CH_2)_s-N(R^{41})(R^{42}) \qquad \text{(VII)}$$

$$-(CH_2)_s-N(R^{43})-(CH_2)_t-N(R^{41})(R^{42}) \qquad \text{(VIII)}$$

$$-(CH_2)_s-N(R^{44})-(CH_2)_t-N(R^{43})-(CH_2)_u-N(R^{41})(R^{42}) \qquad \text{(IX)}$$

$$-(CH_2)_s-N(R^{45})-(CH_2)_t-N(R^{44})-(CH_2)_u-N(R^{43})-(CH_2)_v-N(R^{41})(R^{42}) \qquad \text{(X)}$$

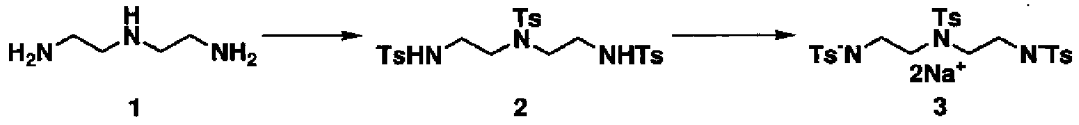
The inside of [type, R^{41}, R^{42}, R^{43}, R^{44}, and a low-grade alkyl group (this amino group -- a low-grade alkyl group.) by which R^{45} was independently replaced by hydrogen atom, a low-grade alkyl group, 1, or two or more amino groups, respectively . It may be replaced by low-grade alkyl sulfonyl group or an aryl sulfonyl group. or -- one -- or -- two -- a piece -- more than -- a hydroxyl group -- replacing -- having had -- low-grade -- an alkyl group -- being shown --; -- s -- t -- u -- and -- v -- respectively -- independent -- two -- or -- three -- an integer -- being shown --] -- expressing -- having -- a basis -- and -- a hydrogen atom -- from -- becoming -- a group -- from -- choosing -- having -- a substituent -- being shown -- although. R^{31} and R^{32} provide a compound expressed with a basis expressed with] which does not show a hydrogen atom simultaneously being shown], or its salt.

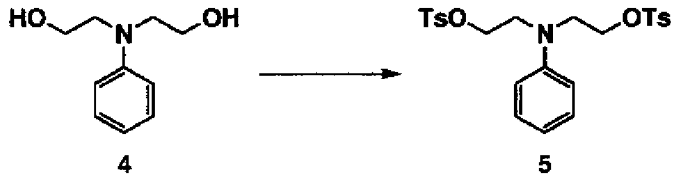
[0009]According to the desirable mode of the above-mentioned invention, (a) R^1, R^3, R^4 and R^6 are hydrogen atoms, R^2 and R^5 are a hydrogen atom or a halogen atom

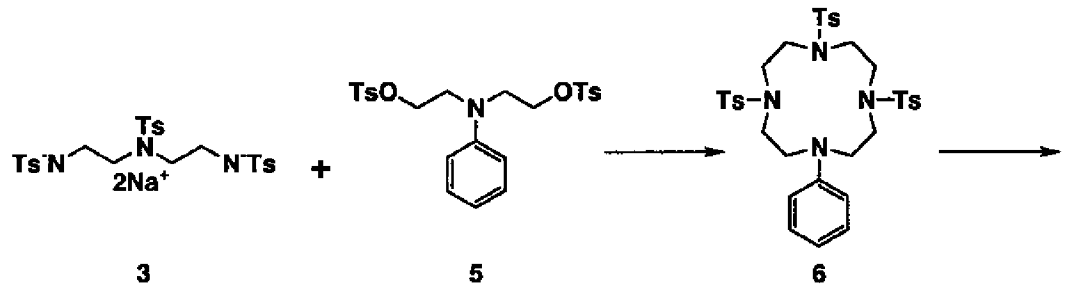
independently, respectively, R^7 and R^8 are hydrogen atoms, and Y is the above-mentioned formula (IV). A compound expressed with formula (I) which is a basis expressed with [the inside of a formula, Z^1, Z^2, and Z^3 are -N(R^{51})- (R^{51} shows a low-grade alkyl group) independently, respectively, and m, n, p, and q are 2], or its salt;
(b) R^1, R^3, R^4, and R^6 are hydrogen atoms, Both R^2 and R^5 are [both] hydrogen atoms, or it is with a halogen atom, The above-mentioned compound which R^7 and R^8 are hydrogen atoms and is a basis as which Y is expressed in the above-mentioned formula (IV) (both the inside of a formula, Z^1, Z^2, and Z^3 are [those with -N(CH_3)-, m, n, p, and q] 2), or its salt;
(c) R^{11}, R^{13}, R^{14}, and R^{16} are hydrogen atoms, R^{12} and R^{15} are a hydrogen atom or a halogen atom independently, respectively, R^{17} and R^{18} are hydrogen atoms, R^{21}, R^{22}, R^{23}, and R^{24} are hydrogen atoms, and Y is the above-mentioned formula (IV). [Z^1, Z^2, and Z^3 are -N(R^{51})- (R^{51} shows a low-grade alkyl group) independently among a formula, respectively, A compound expressed with formula (II) which is a basis expressed with m, n, p, and q being 2] or salt;, and (d) R^{11}, R^{13}, R^{14}, and R^{16} are hydrogen atoms, Both R^{12} and R^{15} are [both] hydrogen atoms, or it is with a halogen atom, The above-mentioned compound which is a basis as which Y is expressed in the above-mentioned formula (IV) (both Z^1, Z^2, and Z^3 are -N(CH_3)- among a formula, and m, n, p, and q are 2), or its salt is provided.
[0010]From another viewpoint, a zinc complex formed from compounds expressed with zinc fluorescent probe; and the above-mentioned formula (I), or formula (II) containing compounds which are the above-mentioned formula (I) or formula (II), and are expressed by this invention, or those salts, or those salts and zinc ion is provided. This zinc fluorescent probe can be used in order to measure an organization and intracellular zinc ion. It is a measuring method of zinc ion by this invention from another viewpoint, How to use compounds expressed with the above-mentioned formula (I) or formula (II), or those salts as a zinc fluorescent probe; It is a measuring method of zinc ion, following process: -- method; including a process to which compounds expressed with (a) above-mentioned type (I) or formula (II), or those salts and zinc ion are made to react, and a process of measuring fluorescence intensity of a zinc complex generated at the (b) above-mentioned process -- and, Use as a compound expressed with the above-mentioned formula (I) or formula (II) or a zinc fluorescent probe of those salts is provided.
[0011]
[Embodiment of the Invention]In calling it a "low-grade alkyl group" in this specification, 1-6 carbon numbers mean the alkyl group which consists of the straight chain of 1-4 carbon numbers, a branched chain, annular, or those combination preferably, for example. As a low-grade alkyl group, more specifically A methyl group, an ethyl group, n-propyl group, An isopropyl group, a cyclopropyl group, n-butyl group, a sec-butyl group, an isobutyl group, a tert-butyl group, a cyclopropyl methyl group, n-pentyl group, n-hexyl group, etc. can be used. When calling it a halogen atom in this specification, they may be any of a fluorine atom, a chlorine atom, a bromine atom, or iodine atoms.
[0012]In the compound of formula (I), R^1, R^3, R^4, And R^6 is a hydrogen atom and R^7 and R^8 are hydrogen atoms, Although it is preferred that R^2 and R^5 are a hydrogen atom or a halogen atom independently, respectively, it is more preferred that both R^2 and R^5 are hydrogen atoms, or both R^2 and R^5 are halogen atoms.

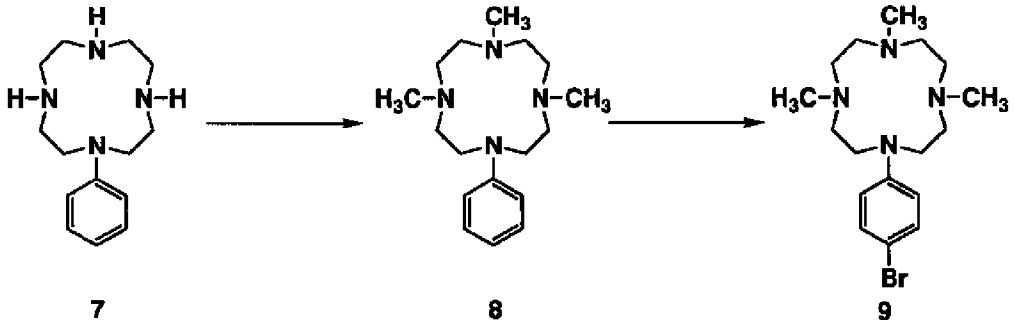
[0013]In the compound of formula (II), R^{11}, R^{13}, R^{14}, And R^{16} is a hydrogen atom and R^{17} and R^{18} are hydrogen atoms, R^{21}, R^{22}, R^{23}, and R^{24} are hydrogen atoms, Although it is preferred that R^{12} and R^{15} are a hydrogen atom or a halogen atom independently, respectively, it is more preferred that both R^{12} and R^{15} are hydrogen atoms, or both R^{12} and R^{15} are halogen atoms.
[0014]As a basis which Y shows, the basis expressed with formula (VI) besides the annular crown residue expressed with the formula of either above formula (III) thru/or (V) can be used. Although Z^1, Z^2, Z^3, and Z^4 show -N(R^{51})-, -O-, or -S- independently, respectively, the low-grade alkyl group (this amino group -- a low-grade alkyl group.) by which R^{51} was replaced by the hydrogen atom, a low-grade alkyl group, 1, or two or more amino groups The low-grade alkyl group which may be replaced by the low-grade alkyl sulfonyl group or the aryl sulfonyl group, or was replaced with 1 or two or more hydroxyl groups is shown. At least one of Z^1, Z^2, Z^3, and Z^4 shows -N(R^{51})-. When two or more [of Z^1, Z^2, Z^3, and the Z^4] are -N(R^{51})-, R^{51} may be the same or may differ. The case where Z^1, Z^2, Z^3, and Z^4 are -N(R^{51})- (R^{51} is an alkyl group) independently is [among these] preferred. As for R51, it is preferred that it is a low-grade alkyl group, this alkyl group may be the same, or it may differ, and it is still more preferred that it is a methyl group.
[0015]It is more desirable to have one amino group at the end of an alkyl group, although the low-grade alkyl group replaced by the amino group which R^{51} shows may have one piece or two amino groups or more in the arbitrary positions of the low-grade alkyl group explained above. This amino group may be replaced by one low-grade alkyl group or two alkyl groups which are the same or are different. It may be replaced by the low-grade alkyl sulfonyl group or the aryl sulfonyl group. As an aryl sulfonyl group, the naphthalene sulfonyl group etc. which is not replaced [the benzenesulfonyl group which is not replaced / substitution or / substitution, or] can be mentioned. Although the number of the substituent on an aryl group, a kind, and replacement positions in particular are not limited, as a substituent, the alkoxyl group of a low-grade alkyl group, a halogen atom, C_1 - C_6, a hydroxyl group, etc. can be mentioned, for example. As for the low-grade alkyl group replaced with the hydroxyl group which may exist on a nitrogen atom, although the carbon number may have one piece or two hydroxyl groups or more in the arbitrary positions of two or more low-grade alkyl groups, it is preferred to have one hydroxyl group at the end of an alkyl group.
[0016]The low-grade alkyl group replaced by the amino group which R^{41}, R^{42}, R^{43}, R^{44}, and R^{45} show, or the low-grade alkyl group replaced with the hydroxyl group can use what was explained above. It is preferred that it is a basis expressed with above formula (IV) as Y, and it is still more preferred that both Z^1, Z^2, and Z^3 are -N(CH_3)-.
[0017]The compound of this invention expressed with formula (I) or formula (II) can exist as acid addition salt or base addition salt. As acid addition salt, for example Mineral acid salt, such as a hydrochloride, sulfate, and a nitrate, Or can mention organic acid salt, such as methanesulfon acid chloride, a p-toluenesulfonic-acid salt, an oxalate, citrate, and a tartrate, etc., and as base addition salt, Organic amine salt, such as metal salt, such as sodium salt, potassium salt, calcium salt, and magnesium salt, ammonium salt, or a triethylamine salt, etc. can be mentioned. A salt with amino acid, such as a glycine besides these, may be formed. Although the compound of this invention or its salt may exist as a hydrate or solvate, each of these substances is included by the range of this

invention.
[0018]According to the kind of substituent, although the compound of this invention expressed with formula (I) or formula (II) may have one piece or two asymmetrical carbon or more, Each of arbitrary mixtures of a stereoisomeric form besides the stereoisomeric form of the optically active substance based on one piece or two asymmetrical carbon or more, the diastereoisomer based on two or more asymmetrical carbon, etc., racemate, etc. is included by the range of this invention. Although a tautomer may exist, it cannot be overemphasized that each tautomer is included by the range of this invention.
[0019]The manufacturing method of the typical compound of the compound of this invention is shown in the following scheme. The manufacturing method indicated to this scheme is shown in the example of this specification in detail and concretely. Therefore, the person skilled in the art can manufacture each compound of this invention expressed with formula (I) or formula (II) by choosing suitably reaction raw materials, a reaction condition, a reaction reagent, etc. based on these explanation, and adding ornamentation and change to these methods if needed. The compounds 2 and 3 indicated in the following scheme are indicated to Org. Synth., 58, 86, and 1979, and the compounds 11, 12, and 13 are indicated to J. Chem. Soc. (Lond.), 3982, and 1955. The compounds 16, 17, and 18 are indicated to Proc. Indian Acad. Sci. Sect. A, 57, 280, and 1963, The compounds 14 and 19 are indicated to J. Biol. Chem., 264, 8171, and 1989, and the compound 22 is indicated to Ber.Dtsch. Chem. Ges., 46.1931-1943, and 1913.
[0020]
[Formula 8]

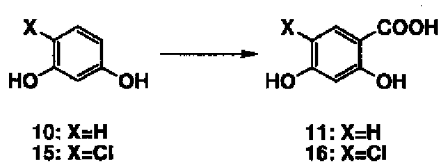






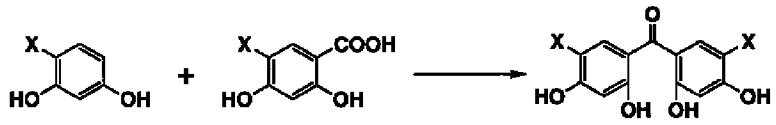


[0021]
[Formula 9]



10: X=H
15: X=Cl

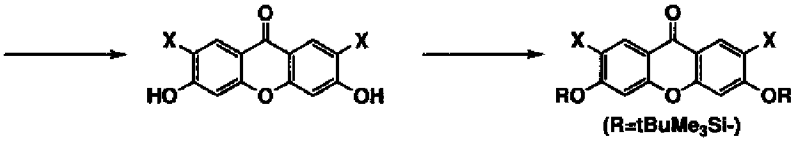
11: X=H
16: X=Cl



10: X=H
15: X=Cl

11: X=H
16: X=Cl

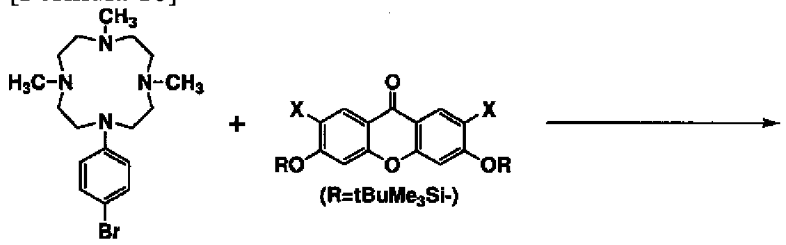
12: X=H
17: X=Cl



13: X=H
18: X=Cl

14: X=H
19: X=Cl

[0022]
[Formula 10]



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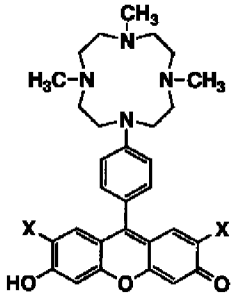
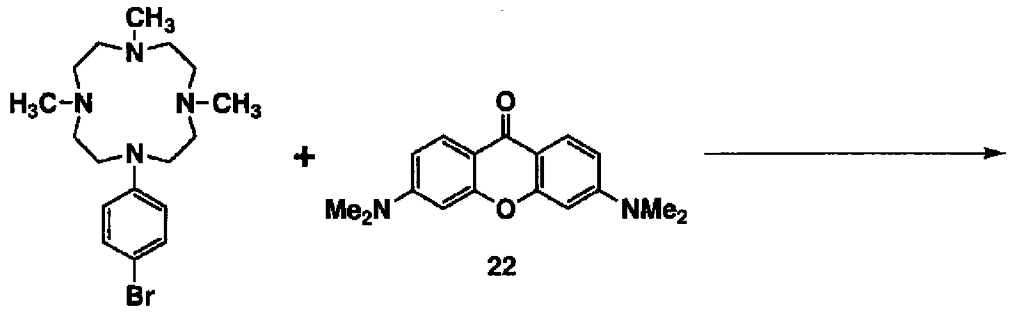
14: X=H
19: X=Cl

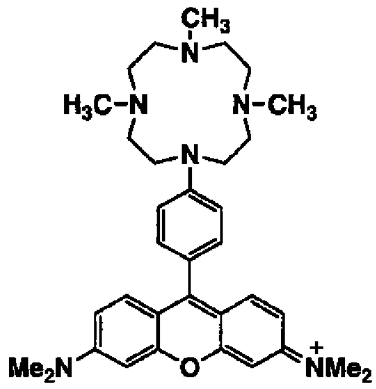


20: X=H (TM12N4-X)
21: X=Cl(TM12N4-X-2)

[0023]
[Formula 11]



9 22



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[0024]The compounds or those salts of this invention expressed with formula (I) or formula (II) are useful as a zinc fluorescent probe. Although the compound of this invention does not have in itself the character which shows a strong fluorescence, if it catches zinc ion and forms a zinc complex, it will come to show a strong fluorescence. Since the compound of this invention shows a strong fluorescence by the excitation light of the long wavelength field which can catch zinc ion specifically and produces an obstacle into neither a body tissue nor a cell, it is very useful as a zinc fluorescent probe for measuring a viable cell and the zinc ion under raw organization under physiology conditions. About the term of "measurement" used in this specification, a fixed quantity should be most interpreted in a broad sense including quality.

[0025]It is possible for the directions in particular for a zinc fluorescent probe of this invention not to be limited, but to use conventionally like a publicly known zinc probe. Usually, aquosity media, such as a physiological saline and buffer solution, or ethanol, acetone, A substance of 1 chosen from a group which consists of compounds expressed with formula (I) and formula (II) by mixture of an organic solvent of water mixing nature, such as ethylene glycol, dimethyl sulfoxide, and dimethylformamide, and an aquosity medium, etc. and those salts is dissolved, What is necessary is to add this solution in suitable buffer solution including a cell and tissue, and just to measure a fluorescence spectrum. For example, the compounds 20 and 21 in the above-mentioned scheme can measure zinc ion of concentration below 10microM, when 495 nm and 505 nm, and a fluorescence wavelength are 515 nm and 525 nm and excited wavelengths use by concentration about 1-10microM, respectively. A zinc fluorescent probe of this

invention may be used with a gestalt of a constituent combining a suitable additive. For example, it is combinable with additives, such as a buffer, a solubilizing agent, and a pH regulator.

[0026]

[Example]Hereafter, although an example explains this invention still more concretely, the range of this invention is not limited to the following example. The compound number in an example is equivalent to the compound number in the above-mentioned scheme.

Example 1: manufacture N-phenyldiethanolamine (4) of the compound of this invention In the solution which dissolved in the pyridine of 100 ml, 15.3 g (84.4 mmol). 70 p-tosylchloride which dissolved in the pyridine of ml It was dropped under ice-cooling, having bet 33.9 g (178 mmol) for 30 minutes. It agitates under ice-cooling after dropping for 2 hours.

Then, the water of 200 ml was added to reaction mixture, and it agitated all night. The depositing solid was separated, it dissolved in the methylene chloride, and this solution was dried with sodium sulfate after washing with water and a saturation salt solution. Decompression distilling off of the solvent is carried out, and it is a compound (5). 37.5 g was obtained. Light yellow solid. 90.8% of yield.

^{1}H-NMR (300 MHz) $CDCl_3$. :. delta2.42 (s, 6H), 3.56 (t, 4H, J=6.0Hz), 4.10 (t,4H, J=6.0Hz), 6.49 (d, 2H, J=8.1Hz), 6.74 (t, 1H, J=7.3Hz), and 7.15 (m, 2H), 7.27 (d, 4H, J= 8.2 Hz) 7.70 MS(d, 4H, J= 8.2 Hz) (FAB): 490(M^++1)m.p.:89 ** (diethylether)

[0027]Compound (3) 41.3 g (67.8 mmol) The solution which dissolved in the anhydrous dimethylformamide of 300 ml, Compound (5) which heated to 100 ** under argon and dissolved in the anhydrous dimethylformamide of 120 ml 36.5 g (74.6 mmol) was dropped over 1.5 hours. After dropping and after agitating at 100 ** for 1 hour and cooling reaction mixture to a room temperature, the water of 500 ml was added and it agitated under ice-cooling for 4 hours. The depositing solid was separated, it dissolved in the methylene chloride, and this solution was dried with sodium sulfate after washing with water and a saturation salt solution. Decompression distilling off of the solvent was carried out, and the rough compound (6) was obtained. A silica gel column refines and it is a compound (6). 29.9 g was obtained. White solid. Yield 62.1%.

^{1}H-NMR (300 MHz) $CDCl_3$:. delta 2.44 (s, 9H), 3.22 (m, 4H), 3.31 (m, 4H), 3.39(t,4H,J=5.0Hz), 3.75 (t, 4H, J=5.0Hz), 6.79-6.74 (m, 3H), 7.23 (m, 1H),7. (d, 2H, J= 8.4 Hz) 32 7.33 (d, 4H, J= 8.1 Hz), 7.68 (d, 4H, J= 8.1 Hz). 7.69 (d, 2H, J= 8.4 Hz) MS(FAB): 711(M^++1)Anal. Calcd for $C_{35}H_{42}N_4O_6S_3$: C and 59.13; H and 5.95; . N and 7.88. Found : C and 58.57; H and 5.84; N and 7.88.m.p.178 ** (methanol)

[0028]Compound (6) broken finely 28.3 g (39.8 mmol) is added to anhydrous n-butanol 900 ml, and it continues, and is sodium. 40 g (1.74 mol) was added, and it flowed back until the piece of sodium disappeared. up to a room temperature -- after cooling -- furthermore -- Sodium of 40 g (1.74 mol) was added, and it flowed back until the piece of sodium disappeared again. 1 l. of water was added little by little under ice-cooling after cooling to the room temperature. After washing n-butanol layer by 0.5N sodium hydroxide, 2N chloride extracted it. After washing 2N chloride layer by diethylether, sodium hydroxide was added, it was made alkalinity and the methylene chloride extracted. After the saturation salt solution washed the organic layer, it dried with potassium carbonate. Decompression distilling off of the solvent is carried out, and it is a

compound (7). 6.60 g was obtained. Brown oil. 66.8% of yield.
^{1}H-NMR (300 MHz) $CDCl_3$:. delta 2.63 (t, 4H, J=4.8Hz), 2.77-2.84 (m, 8H), 3.38(t, 4H, J=5.0Hz), 6.82 (t, 1H, J=7.3Hz), 6.98 (d, 2H, J=8.1Hz, 7.23 (m, 2H) MS(FAB): 249 (M^++1) [0029]Compound (7) It is methanol about 5.00 g (20.2 mmol). It melts in 300 ml and is formaldehyde fluid 37%. 50 ml is added, and it continues and is cyano hydrogenation boron sodium. 13.4 g (213 mmol) was added little by little. After agitating at a room temperature for 2 hours, acetic acid was added, the pH of the solution was made into neutrality, and it agitated at the room temperature further for 3 hours. Decompression distilling off of the methanol was carried out, it was suspended in 2N sodium hydroxide solution, and the methylene chloride extracted residue. After washing a methylene chloride layer by 0.5N sodium hydroxide, 2N chloride extracted. Sodium hydroxide was added to 2N chloride layer, it was made alkalinity, and the methylene chloride extracted. The methylene chloride layer was dried with potassium carbonate after washing with the saturation salt solution. The methylene chloride was distilled off under decompression and the rough compound (8) was obtained. An alumina column refines and it is a compound (8). 1.53 g was obtained. Brown oil. 26.1% of yield.
^{1}H-NMR (300 MHz) $CDCl_3$:. delta 2.25 (s, 3H), 2.31 (s, 6H), 2.53 (m, 8H), 2.80(t, 4H, J=5.9Hz), 3.51 (t, 4H, J=5.9Hz), 6.63 (m, 3H),7.19 (dd and 2H.) J= 7.2 and 8.8HzMS (EI): 290 (M^+) [0030]Compound (8) It is dioxane about 501 mg (1.73 mmol). 20 ml and water 0.38 ml added 5N potassium hydroxide solution to the solution which dissolved in 10 ml. The bottom of ice-cooling, bromine It is dioxane about 105 ml (2.04 mmol). The solution which dissolved in 8 ml was dropped over 2 hours, and reaction mixture was agitated under ice-cooling after dropping for 1 hour. Decompression distilling off of the solvent was carried out, and residue was suspended to the methylene chloride. After washing a methylene chloride layer by 0.5N sodium hydroxide, 2N chloride extracted. After washing 2N chloride layer by diethylether, sodium hydroxide was added, it was made alkalinity and the methylene chloride extracted. After the saturation salt solution washed the methylene chloride layer, it dried with potassium carbonate. Decompression distilling off of the methylene chloride is carried out, and it is a compound (9). 572 mg was obtained. Brown oil. 89.6% of yield.
^{1}H-NMR (300 MHz) $CDCl_3$:. delta2.17-2.23 (m, 8H), 2.43 (s, 3H), 2.44 (s, 6H),2.69 (t, 4H, J=6.0Hz), 3.39 (t, 4H, J=6.0Hz), 6.44 (d, 2H, J= 9.2 Hz, 7.17 (d, 2H, J= 9.2 Hz) MS(EI^+): 368 and 370 (M(1:1) $^+$) [0031]Compound (9) It is anhydrous 2-methyltetrahydrofuran about 299 mg (0.81 mmol). In addition to 20 ml, it cooled to -150 ** in the bottom liquid nitrogen of argon-isopentane bath, and 1.64N tert-butyl lithium n-pentane solution 2.85 ml was added. Compound (14) after checking disappearance of a raw material by TLC The solution which dissolved 719 mg (1.58 mmol) in the tetrahydrofuran of 25 ml was added little by little. -After agitating at 150 ** for 1 hour, reaction mixture was returned to the room temperature. After adding the mixed liquor of water 20 ml and tetrahydrofuran 10 ml to reaction mixture, decompression distilling off of the solvent was carried out. It is 2N chloride to residue. 10 ml was added, and it shaded and agitated at the room temperature for 1 hour. After washing the solution of hydrochloric acid by diethylether, sodium hydroxide was added, it was made alkalinity and the water layer was again washed by diethylether. pH was adjusted to 7-8 using 2N chloride and 2N sodium hydroxide, the solid which has deposited was separated, and the rough compound (20) was obtained. An octadecyl column refines and it is a compound

(20). 90.7 mg was obtained. Brown solid. 22.4% of yield.
^{1}H-NMR (300 MHz) DMSO-d_6+D_2O:. delta 2.16 (s, 3H), 2.61 (m, 10H), 2.85 (m, 4H), 2.99 (m, 4H), 3.72 (m, 4H), 6.28 (d, 2H, J=2.0Hz), 6.37 (dd, 2H, J= 2.0, 9.3 Hz, 6.87 (d, 2H, J= 8.8 Hz), 7.04 (d, 2H, J= 9.3 Hz), 7.27 MS (d, 2H, J= 8.6 Hz) (FAB) : 501(M^++1)m.p.:189 ** [0032]Compound (9) It is anhydrous 2-methyltetrahydrofuran about 300 mg (0.81 mmol). In addition to 30 ml, it cools to -150 ** in a bottom liquid nitrogen of argon-isopentane bath, and is a 1.54N t-butyl lithium n-pentane solution. 2.50 ml was added. Compound (19) after checking disappearance of a raw material by TLC The solution which dissolved 500 mg (0.95 mmol) in the tetrahydrofuran of 10 ml was added little by little. -Reaction mixture was returned to the room temperature after 1-hour churning at 150 **. They are water 20 ml and a tetrahydrofuran to reaction mixture. After adding the mixed liquor of 10 ml, decompression distilling off of the solvent was carried out. It is 2N chloride to residue. 10 ml was added, and it shaded and agitated under the room temperature for 1 hour. After washing the solution of hydrochloric acid by diethylether, sodium hydroxide was added, it was made alkalinity and methylene chloride methanol (5:1) extracted. The organic layer was dried with sodium sulfate after washing with the saturation salt solution, decompression distilling off of the solvent was carried out, and the rough compound (21) was obtained. The octadecyl column refined and the compound (21) was obtained. Brown solid. 1.2% of yield.
^{1}H-NMR (300 MHz) CD_3OD: delta 2.26 (s, 3H), 2.66-2.69 (m, 4H), 2.68 (s, 6H),2.89-2.93 (m, 4H), 3.09 (m, 4H), 3.79 (m, 4H), 6.54 (s, 2H, 6.96 (d, 2H, J= 8.8 Hz) 7.29 (s, 2H), 7.31 MS (d, 2H, J= 8.8 Hz) (FAB) 569, 571, 573 m(M^++1).p.:230 **
[0033]Example 2: the selectivity to zinc ion was evaluated using the compound 20 and the compound 21 which were obtained in the selectivity above-mentioned example 1 over zinc ion. 100 mM HEPES containing various metal ions (5microM or 5 mM) The compound 20 of 5microM or 21 was added to inside (pH 7.5), and fluorescence intensity was measured. About the compound 20, they are excited wavelengths. 495 nm, fluorescence wavelength It is referred to as 515 nm and they are excited wavelengths about the compound 21. 505 nm, fluorescence wavelength The fluorescence spectrum was measured as 525 nm. A result is shown in drawing 1 and drawing 2. Among a figure, the fluorescence intensity of a vertical axis sets fluorescence intensity when not adding the metal ion to 1, and shows fluorescence intensity when each metal ion is added numerically. Under existence of the sodium ion to which the compound of this invention has very high selectivity to zinc ion, and exists so much in the living body, potassium ion, calcium ion, etc., it is clear from these results that fluorescence intensity does not increase at all.
[0034]Example 3: as a detection sensitivity zinc fluorescent probe of a zinc fluorescent probe. Newport Green (Handbook of FluorescentProbes and Research Chemicals,6th Edition by Richard P. Haugland, pp.531-540 currently used. The sensitometry of the zinc fluorescent probe of this invention was measured. 100 mM HEPES which contains the zinc ion of various concentration (to 10microM) The compound 20 of 5microM, the compound 21, or Newport Green was added to inside (pH 7.5), and fluorescence intensity was measured. About the compound 20, they are excited wavelengths. 495 nm, fluorescence wavelength It is referred to as 515 nm, About the compound 21, they are excited wavelengths. 505 nm, fluorescence wavelength It is referred to as 525 nm and they are excited wavelengths about Newport Green. 505 nm, fluorescence wavelength

The fluorescence spectrum was measured as 530 nm. A result is shown in drawing 3. Among a figure, the fluorescence intensity of a vertical axis sets fluorescence intensity when not adding the metal ion to 1, and shows fluorescence intensity when each metal ion is added numerically. The compound of this invention has far high detection sensitivity compared with Newport Green conventionally used as a zinc fluorescent probe so that clearly from this result.

[0035]Example 4: about the fluorescence intensity compounds 20 and 21 of the zinc fluorescent probe, the correlation of zinc ion concentration and fluorescence intensity was investigated. 100 mM CAPS which contains the zinc ion of various concentration (to 10microM) The compound 20 or the compound 21 of 5microM was added to inside (pH 10.0), and fluorescence intensity was measured. About the compound 20, they are excited-wavelengths 495 nm and a fluorescence wavelength. It is referred to as 515 nm and they are excited wavelengths about the compound 21. 505 nm, fluorescence wavelength The fluorescence spectrum was measured as 525 nm. A result is shown in drawing 4 and 5. Fluorescence intensity rose depending on the concentration of zinc ion in both compounds, and it was accepted under existence of zinc ion of 1 Eq or more to the compound that fluorescence intensity becomes fixed. This result shows that the compound of this invention forms zinc and the complex of 1:1.

[Translation done.]